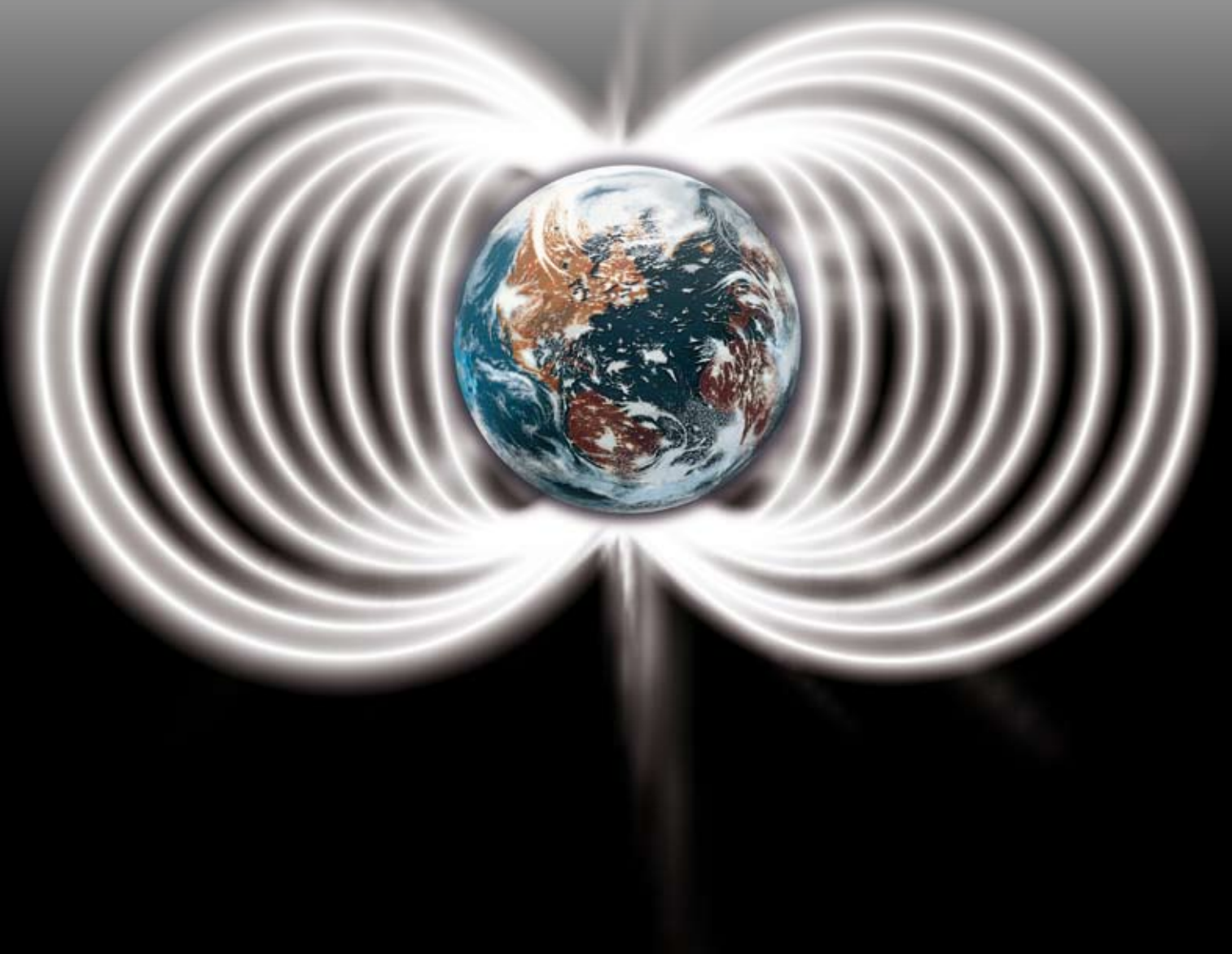


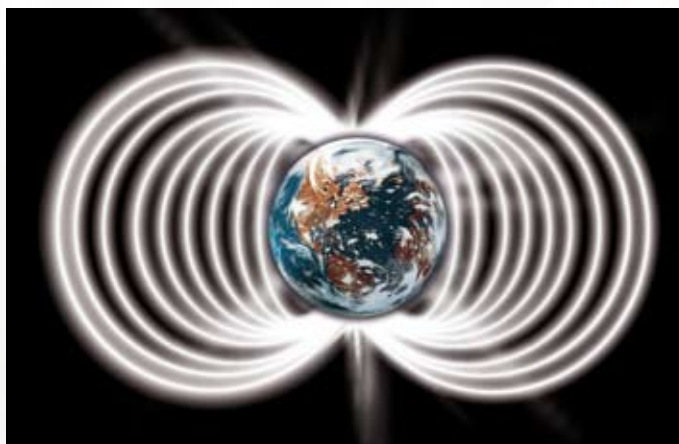
YMC HILIC Columns for Polar Analytes



Polar Analytes

Advantages

In general, reliable applications in HPLC require a minimum retention factor (k') of 2. In the widely used reversed phase methods polar compounds such as peptides, organic acids or pyrimidines often exhibit little or no retention and therefore low resolution on a conventional C18 phase. The use of additives such as ion-pair reagents is not an alternative since it is not applicable to LC-MS. Also normal phase chromatography fails due to poor solubility of the polar compounds in organic solvents. HILIC fills the gap between reversed phase and normal phase chromatography, because polar compounds can be retained while using e.g. acetonitrile/water eluents and buffers suitable for LC-MS.

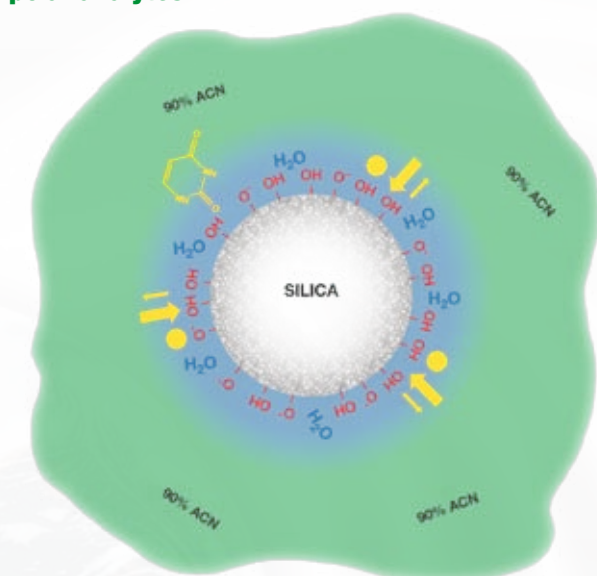


Polar Analytes

Hydrophilic Interaction Chromatography (HILIC)

Hydrophilic Interaction Chromatography is a technique which has attracted more and more attention since it offers an alternative approach for the separation of highly polar compounds. The method itself, although it has been known for more than 25 years, has become more popular recently due to the introduction of several specialised HILIC selectivities onto the market. However it is not well known that HILIC can be accomplished with any highly polar stationary phase.

**High organic modifier concentration:
strong interaction between stationary phase and polar analytes**



● = polar compound

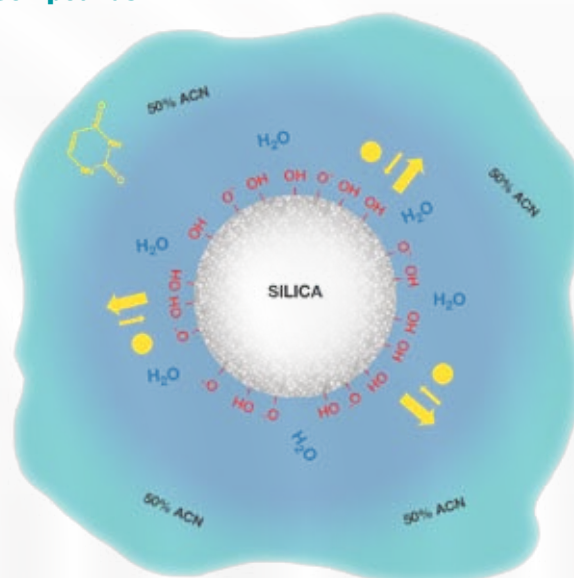
HILIC is a very interesting mechanism since any standard reversed phase material hardly can retain polar, water-soluble compounds and an alternate mode of retention and separation needs to be considered for very polar compounds such as pharmaceutical actives, metabolites and peptides. Hydrophilic Interaction Chromatography has also been referred to as “aqueous normal phase” or “reverse reversed phase” since elution is in the order of increasing hydrophilicity and the organic portion of the mobile phase (typically acetonitrile) is the weak solvent and the aqueous portion is the strong eluting solvent.

HILIC columns from YMC are rugged stationary phases which provide improved LC/ESI-MS response, direct SPE solvent compatibility and complementary selectivity to reversed phase. This is important to R&D and drug metabolism scientists since the impurity or metabolite is frequently more polar and present at much lower concentrations than the parent compound. With YMC HILIC columns, these very polar compounds elute later than the higher hydrophobic parent compound, thereby minimising the MS ion suppression that can occur at the beginning of the chromatogram.

This opens up a large range of materials which are suitable for HILIC separations. In order to understand the mechanism and the separation principle it is helpful to use a schematic drawing:

As shown below, HILIC uses a highly polar stationary phase and a non polar mobile phase e.g. functionalised silica with a hydrophilic coating and an acetonitrile/water mixture (80/20). The polar or hydrophilic sample experiences more attraction towards the stationary phase or the more polar and more stationary part of the mobile phase. Polar samples can be eluted by using isocratic or gradient methods, giving the mobile phase a more hydrophilic character or even a higher ionic strength.

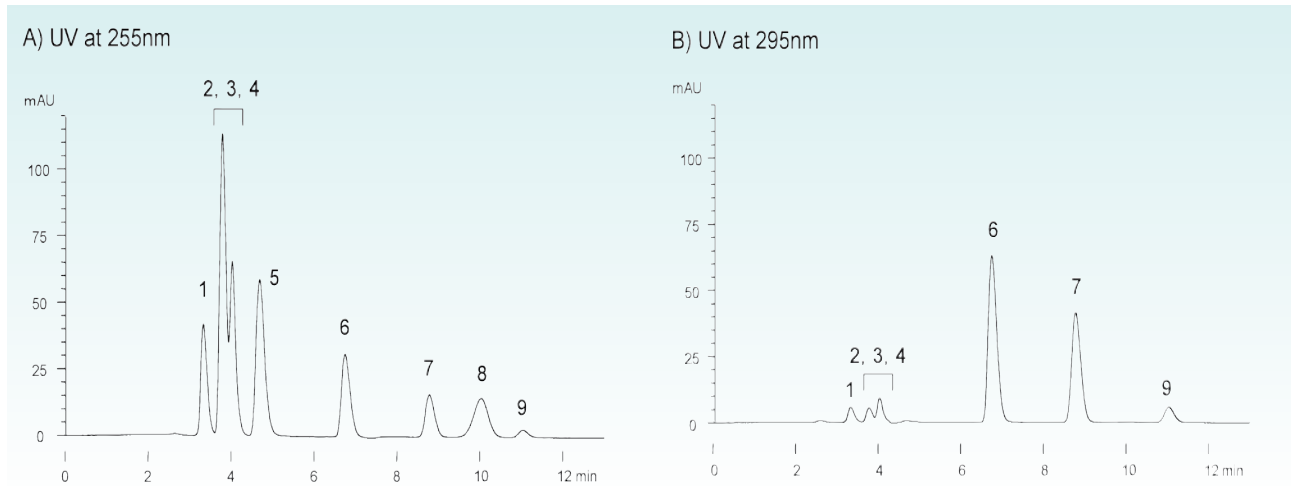
Increasing the aqueous content decreases the interaction between stationary phase and polar compounds



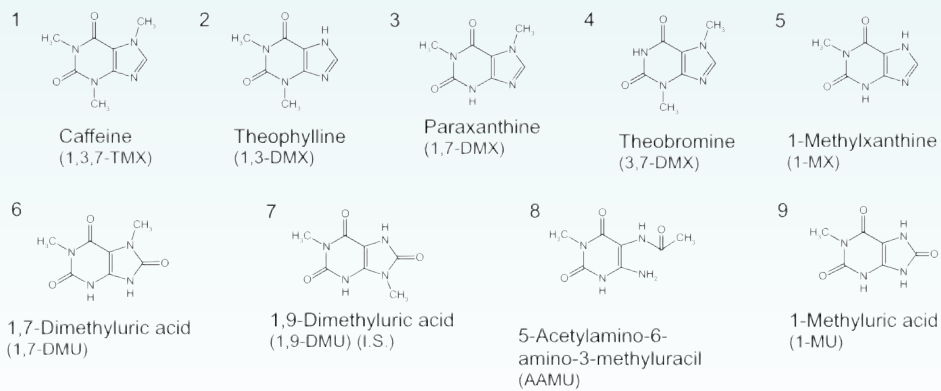
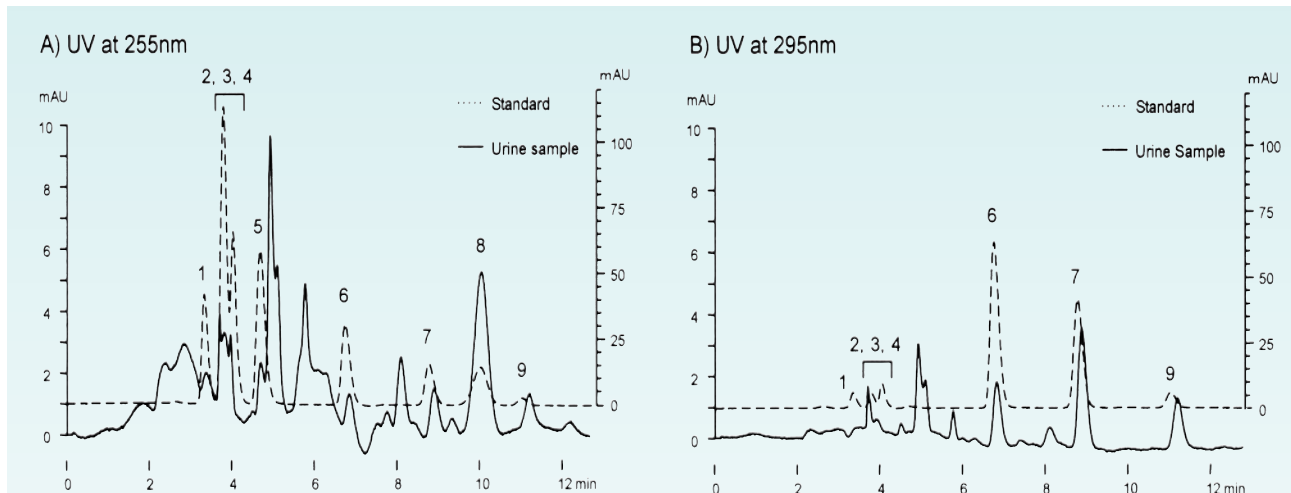
- YMC HILIC provides an ideal mechanism for the analysis of extremely polar analytes
- YMC offers a wide variety of HILIC phases to cover a broad range of polar selectivities
- HILIC eluents are compatible with LC-MS systems

YMC HILIC Applications

Caffeine metabolites (standard)



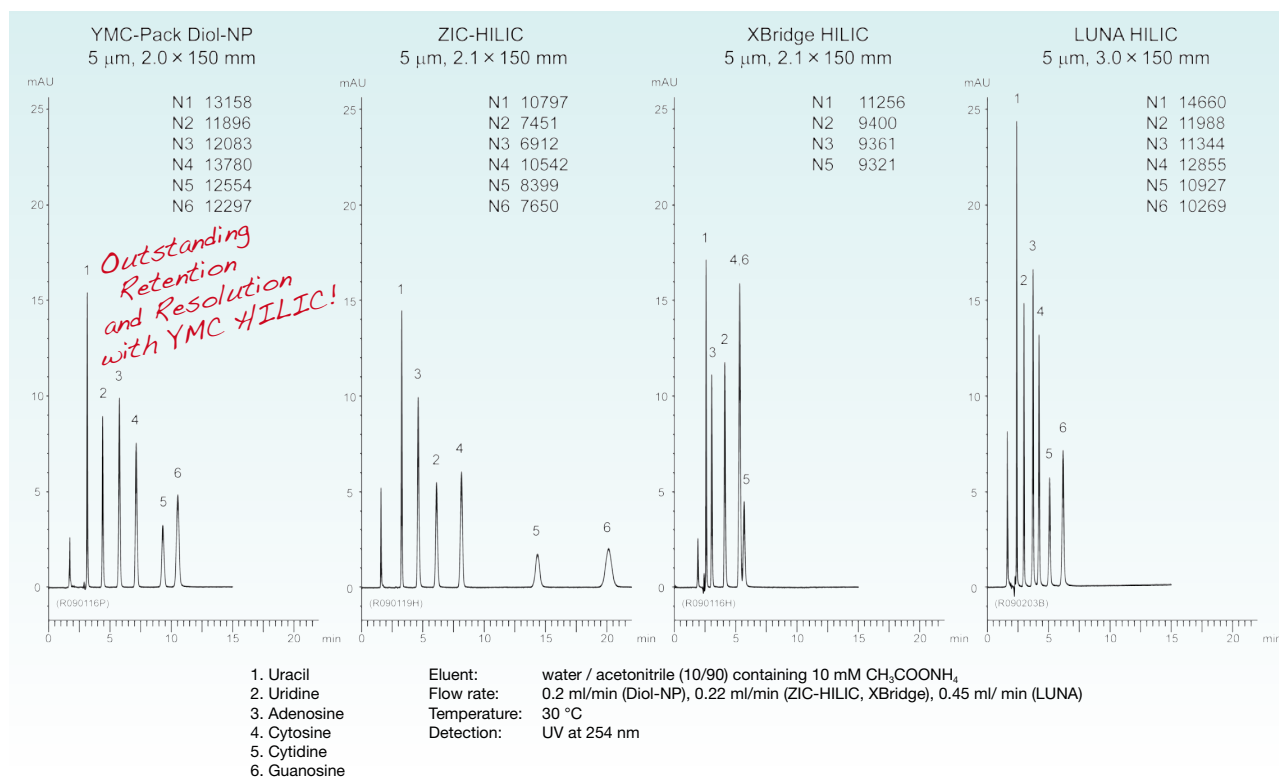
Caffeine metabolites (urine sample)



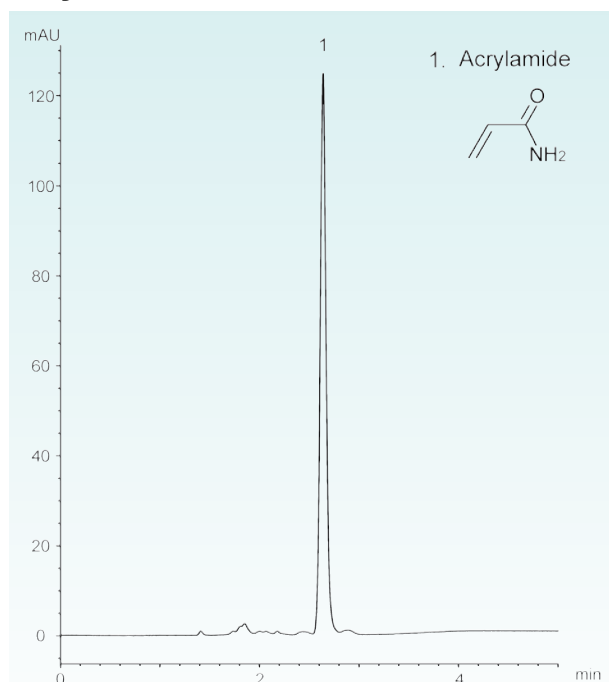
Column: YMC-Pack Polyamine II (5 µm, 12 nm) 250 x 4.6 mm ID
 Eluent: acetonitrile / 0.75% formic acid (4/1)
 Flow rate: 1.0 ml/min
 Temperature: ambient
 Detection: A) UV at 255 nm, B) UV at 295 nm
 Injection: 10 µl

YMC HILIC Applications

Nucleosides and bases



Acrylamide



Column: YMC-Pack Diol-NP (5 μm, 12 nm) 150 × 2.0 mm ID
Eluent: 20 mM CH₃COONH₄ / acetonitrile (5/95)
Flow rate: 0.2 ml/min
Temperature: 25 °C
Detection: UV at 210 nm
Injection: 1 μl (40 μg/ml)

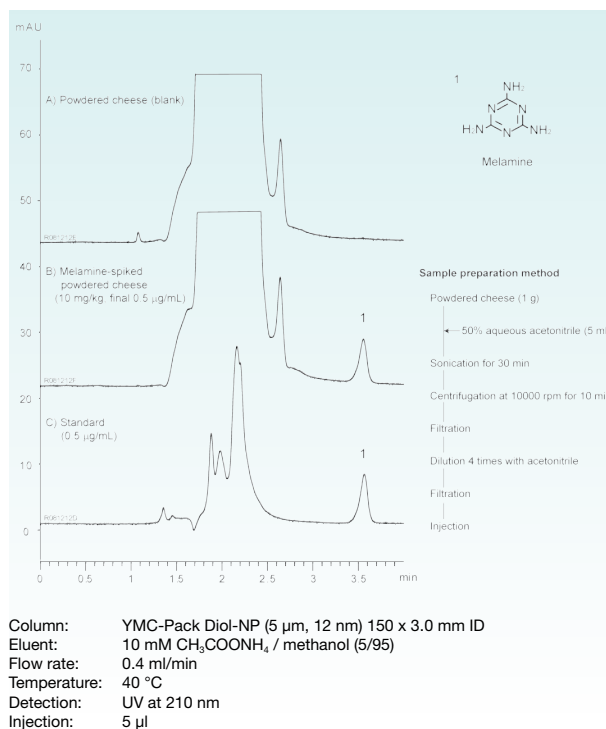
Since the detection of the genotoxic compound, acrylamide in starchy foodstuffs subjected to prolonged heating, the analysis of this compound has been of major interest.

YMC HILIC Applications

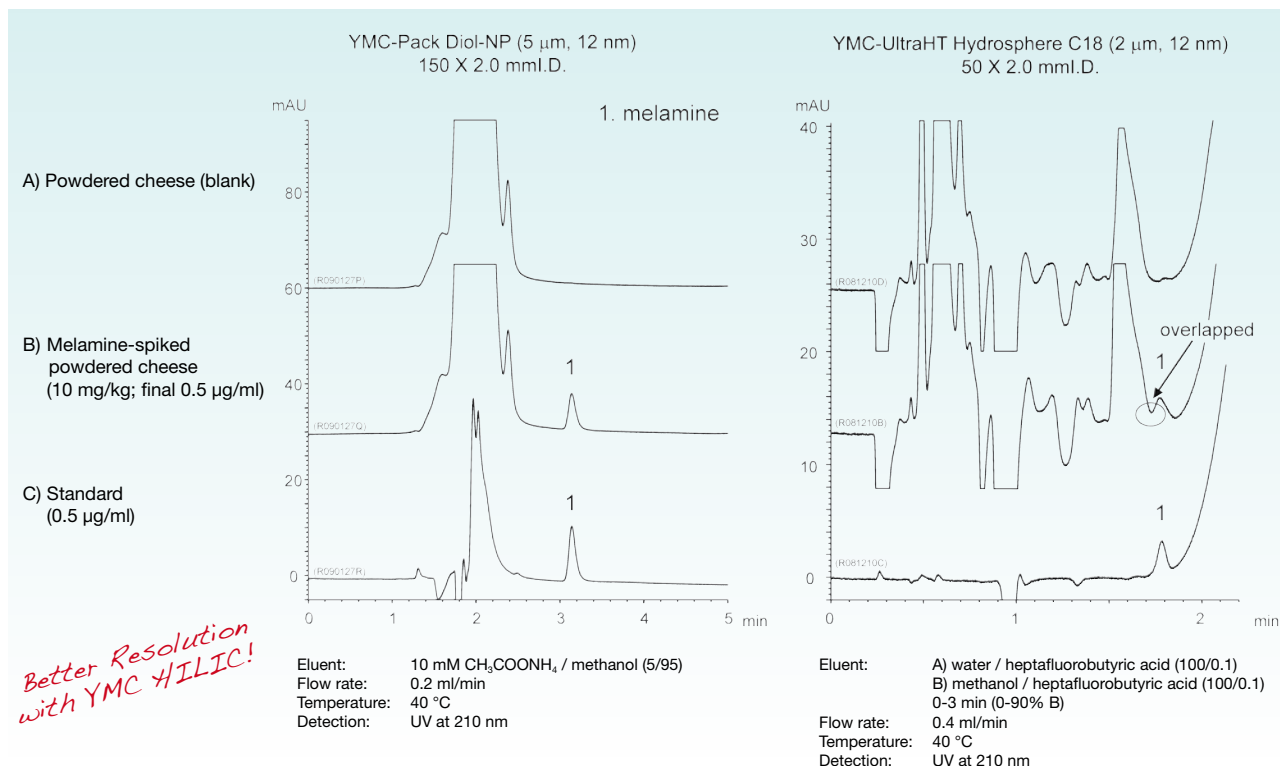
Melamine (sometimes confused with the plant and animal compound, melanin or melanine) is an industrial chemical used in the manufacture of plastic products. Despite that, tests showed that a number of milk companies have used it in processing milk to artificially increase the apparent protein content by addition of non-protein nitrogen and so allowing illegal dilution of the product with water with a corresponding increase in sales value.

The problem is that melamine forms crystals of melamine cyanurate from aqueous solutions when mixed with cyanuric acid (which forms naturally by hydrolysis of melamine). These low solubility, virtually indigestible crystals concentrate in the renal microtubules and form large numbers of round spoke-like, yellow crystals, damage the renal cells and ultimately kidney failure. Recently, more and more attention has been focussed on melamine in milk and food products (including, for example, chocolate). The most distressing incidents occurred in the summer of 2008 when more than 54,000 babies became seriously ill and four died in China due to suspected milk formula. However, earlier in 2007 the FDA reported that an estimated 8,500 cats and dogs died after eating melamine-contaminated pet food. In order to avoid further accidents involving melamine contamination, it is essential to provide an analytical method, which monitors the level of melamine without compromising resolution due to excessive background signals from the sample matrix. YMC have developed an fast HILIC method which also can be transferred to LC/MS detection.

Melamine in powdered cheese

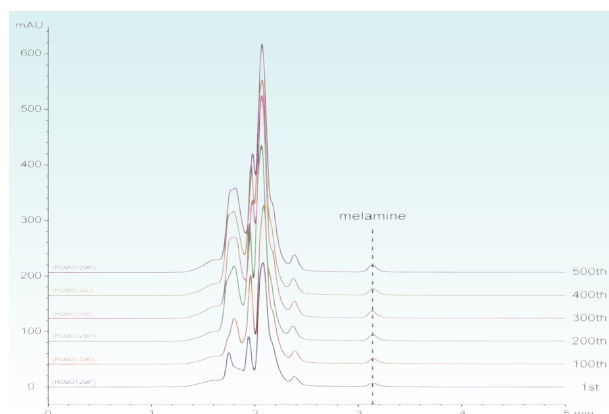


Melamine in dairy products: HILIC versus RP



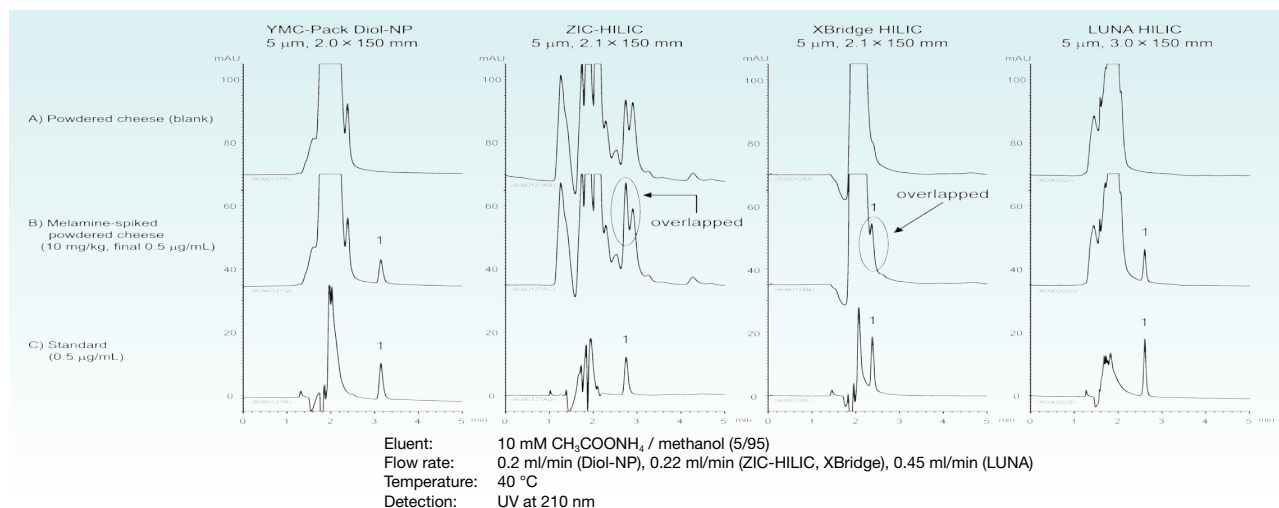
YMC HILIC Applications

Reproducibility of YMC HILIC

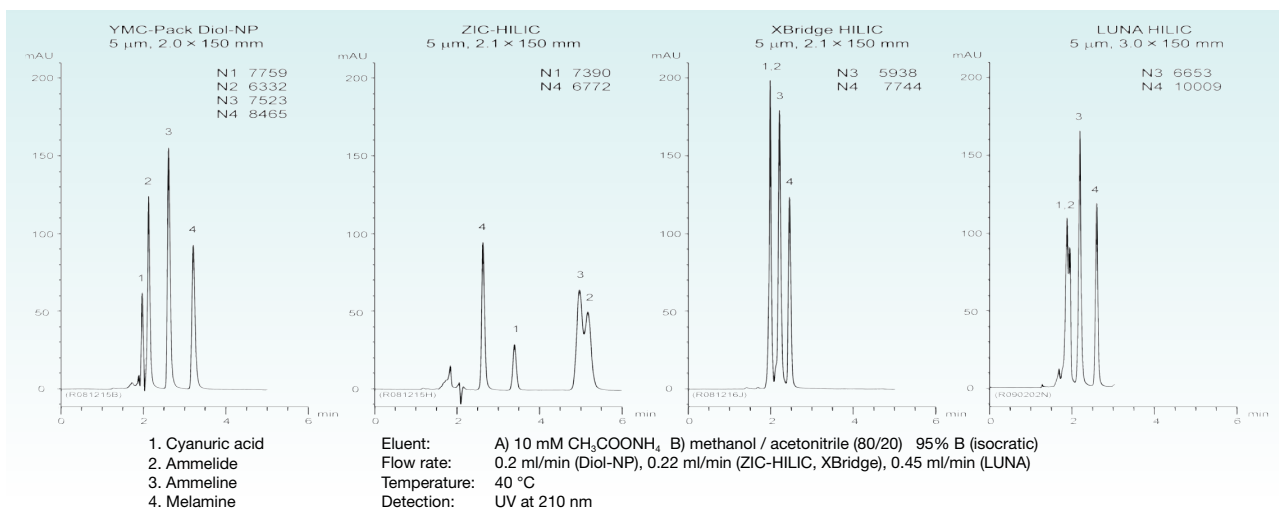


Column: YMC-Pack Diol-NP (5 μ m, 12 nm) 150 x 2.0 mm ID
 Eluent: 10 mM $\text{CH}_3\text{COONH}_4$ / methanol (5/95)
 Flow rate: 0.2 ml/min
 Temperature: 40 $^\circ\text{C}$
 Detection: UV at 210 nm
 Sample: melamine-spiked powdered cheese (0.5 $\mu\text{g}/\text{ml}$)

Comparison of YMC HILIC Diol with other conventional HILIC phases



Comparison of YMC HILIC Diol with other conventional HILIC phases



Ordering information

YMC-Pack Diol, 12 nm, 5 µm

Column length (mm)	Column ID (mm)		
	2.1	3.0	4.6
150	DN12S051502	DN12S051503	DN12S051546
250	DN12S052502	DN12S052503	DN12S052546

YMC-Pack Polyamine II, 12 nm, 5 µm*

Column length (mm)	Column ID (mm)		
	2.1	3.0	4.6
150	PB12S051502	PB12S051503	PB12S051546
250	PB12S052502	PB12S052503	PB12S052546

*YMC-Pack Polyamine II is well known for the analysis of sugars. But YMC-Pack Polyamine II is a polar phase and can also be used in the HILIC mode.

Guard Columns are available in different dimensions.

For more details please contact us: Phone 02064-427-0 or email info@ymc.de.

Order now (info@ymc.de or www.ymc.de):



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Germany
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YMC Co., Ltd.

YMC Karasuma-Gojo Bld. 284 Daigo-cho,
Karasuma Nishiiru Gojo-dori Shimogyo-ku,
Kyoto 600-8106 Japan
TEL. +81(0)75-342-4515, FAX +81(0)75-342-4550
www.ymc.co.jp

YMC America, Inc.

941 Marcon Boulevard Suite 301
Allentown, PA18109 USA
TEL. +1-610-266-8650, FAX +1-610-266-8652
www.ymc-america.com

YMC India Ltd.

CX - 07, 3rd Floor, Lobe - 1,
Tower - A, The Corenthum, Plot No- A-41,
Sector - 62, Noida - 201301 (UP) India.
TEL. +91(0)120-4276020 - 25, FAX +91(0)120-4276026
www.ymcindia.com